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RESEARCH ARTICLE

Rehabilitation of Tindouf argan forest and reforestation outside by seeds propagation and effect of Interactions with mycorrhizal fungi on growth of the argan seedlings (*Argania spinosa* (L.) Skeels) in Algeria

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Abstract:

The Argan tree, being a xérophile species, observed in the semi-arid and dry floor, presents specific ecological characteristics and numerous interests (forest, fodder, and fruit). The study concerns the highlighting of endomycorrhizal associations of the Argan tree (*Argania spinosa* (L.) Skeels) of Tindouf (southwest of Algeria) this tree is an endemic forest gasoline of the Algerian southwest. According to the technique of tint in the acid fuschine indicates the presence of endomycorhiza to vesicles and to arbuscular. The results show an increase of 70.5 ± 1.35 cm from the average stem height, with high average number of branches of 1st and 2nd order reach 23.71 ± 0.97 and 15.37 ± 1, 19 respectively. And 52.36 ± 1.08 cm in average root length, with high average number of secondary roots reach brown 32.56 ± 1.45, Similarly, ground biomass is increased by 11.45 ± 1.47 and root biomass of 7.47 ± 0.52 in plants inoculated (mycorrhizal) against by the non-inoculated control plants were recorded as the values of the low plants inoculated values of the average stem height, average number of branches of 1st and 2nd order, the average root length, the average number of secondary roots, respectively about 40.4 ± 0.44 cm, 07, 65 ± 0.71, 0, 35.58 ± 0.92 cm, 13.83 ± 1.04. Even the root and shoot biomass recorded values within the range of 7.53 ± 0.86 and 4.25 ± 0.48 respectively, The index of relative mycorrhizal dependency (IRMD) is 45.77%. Under the effect of mycorrhiza, the average air ratios parties / party root password from 1.05 to 1.50, indicating a greater efficiency of mycorrhizal root systems. .As for the interest of the mycorrhizal symbiosis to the Argan tree, the mycorhization checked by an origin selected by endomycorrhizal mushroom was translated by a very positive effect on the growth of young seedlings of the Argan tree inoculated by report the not inoculated seedlings.

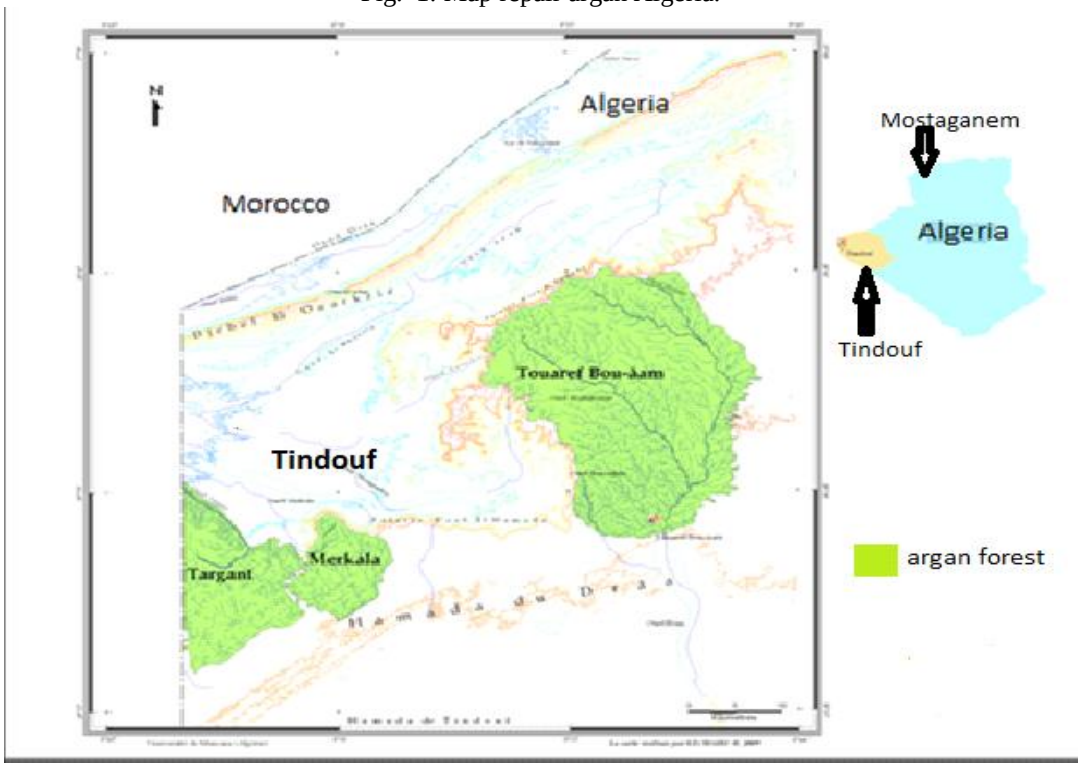
Key Words: Argan tree, mycorrhizal , endomycorrhizal, inoculated, not inoculated, growth.

Introduction

In southern Algeria (Tindouf), forest based on endemic heritage: the argan tree (*Argania spinosa* L. Skeels), which is the only tree ecology triple interest: forest, forage and fruit. The argan tree is met on the margins of Northwest Tindouf. It occupies 90.644 hectar on the sandy wadis, gravel and rocky. The very low slope (0-6%) on Hamada, tree density increases gradually toward the Atlantic Ocean depicting tree monotype type (Kechairi, 2009) (Fig 1). This tree has ecological and physiological properties such that it is virtually the only tree adapted to arid and semi-arid. The argan tree is virtually irreplaceable soil conservation, pasture and the fight against erosion, desertification. With its roots, which can reach several meters in length, this very hardy tree involved in setting it enriches soil organic matter also from its leaves. (Turner, 1990; Balo et *al*., 1995). The study of the morpho behavior - physiological and biochemical plants of the argan tree, bound (connected) to put under water stress show a strong resistance (Belkhodja and Benaouf, 2009). The viability of the forest system is threatened by overexploitation and overgrazing in Tindouf (Touaref bouaama, Targanat and Markala) by the local population and the nomads of Polisario. This results in severe soil degradation and a decrease in oil production (traditional extraction), food béatilles (camels and sheep). It is therefore. National concern for the authorities concerned to protect our rare and vulnerable heritage arganeraie against anthropogenic pressure, allowed the establishment of an Executive Order by the Wali of Tindouf state bearing draft reserve creation natural. Decree No. 04/96 of 12/06/2004 on the protection of plant species argan in the Tindouf state (Kechairi, 2009). Propagation by seed is the most common method used to

reproduce the argan forest species, wholes reforestation projects in Algeria Argan adopted this method, the most successful example of the reforestation project in Mostaganem coast its trees started fruiting after six years, and all nurseries Tindouf aimed rehabilitation of argan. the use of controlled mycorrhiza could probably significantly increase the success of transplants and initial growth of trees as seedlings in vitro argan products have proven to be highly dependent on mycorrhizal symbiosis (Nouaïm and Chaussod, 1994) . The endomycorrhizal symbiosis that is the object of our work concerns the vast majority of plants semi-arid and dry areas and some known as the argan tree that resist drought through endomycorrhizae (Nouaïm et *al*., 1991) trees.

Fig. 1: Map repair argan Algeria.



Material and methods

Mycorrhiza in the argan tree has been observed for the first time in 1988. Taken roots in several places of the argan tree were colonized by endomycorrhizal fungi (Nouaïm, 1994). Plant Material, these seedlings propagated by seeds of argan in the laboratory from adults collected from the subjects arganerais Tindouf July 2007 seeds. And argan mycorrhizal plants collected in the region of Tindouf.

Multiplication endomycorrhizae from the argan tree seedlings mycorrhizal

The argan tree seedlings were weighed and divided into two lots of 100 individuals representing a comparable distribution to form the two treatments. The first is non-inoculated, the second is subject to mycorrhizal inoculation vesicular arbuscular for the demonstration of the mycorrhizal dependency of the argan tree (Nouaïm and Chaussod, 1994), germinated seeds were placed in pots 2 liter culture (diameter 14 cm, height 18 cm) each containing 1100 g (peat + sand). The pots are of limited volume and inoculated plants collect more nutrients, causing a depletion faster than non-inoculated medium. This is why a NPK fertilizer was made. The mycorrhizal inoculation was made from mycorrhizal roots: This technique involves cutting the roots of mycorrhizal seedlings argan from the region of Tindouf in small fragments (5 mm), these fragments are then disinfected with hypochlorite sodium 5% for 3 to 5 minutes, are then crushed in the end they were incubated in soil of pots each abutted our roots. The two lots of seedlings were watered regularly with water was done throughout the experiment.

Methods of macroscopic observation (aerial part and root)

The aerial part has been followed for nine months from February to September. This monitoring was to make monthly measurements on the main axis and branches 1st and 2nd order to study the growth of different plant parts of each question. This was intended to capture the growth in relationship with mycorrhiza (Fig 2).

The observation of the root system of plants is taken from uprooting them. Washing the roots with tap water is needed. In a good light, you can see that the root system has two different aspects: the roots of brown that showed after the observations microscpiques endomycorrhizae presence, which is not the case with the roots of color white, monitoring was to make monthly measurements of the length of the roots with enumeration of secondary roots.

Fig.2: Measurements of air and root Parties argan mycorrhizal seedlings.

The index of relative mycorrhizal dependency

The index of relative mycorrhizal dependency (IRMD) was calculated from the average air and root biomass of plants (dry weight)

IRMD = 100 (+ PSM-PSM-) / PSM +, Where + PSM and PSM-represent respectively the dry weight of mycorrhizal plants and non-mycorrhizal (Plenchette, 1983). The root system was rinsed with distilled water and ultrasonically cleaned to remove debris substrate adhering to them before weighing water. For each plant, after determining the root fresh weight.

Methods for microscopic observation

To achieve these observations, the roots are cut into pieces of 1cm, and then immersed in a water container. This method is to observe with a light microscope in the biology laboratory, semi-finished cuts made longitudinally at the tender roots to short cuts. Subsequently, the roots were stained with acid fuchsin. The chemicals used for coloring (potash (KOH) 10% fuchsin acid powder, Hydrochloric Acid (HCL) 0.1 N, fuchsin acid gives a red color to mycorrhizal cells. Coloring roots method (Kormanik et *al*., 1980), the method involves staining

fuchsin acid the steps:

Wash-root system with tap water.

-Cut the roots into pieces of 1cm.

-Soak the roots in 10% KOH, the bottles are covered with a watch glass and placed in a water bath for one hour at 90 ° C.

-Rinse roots aves water.

- Acidification in N/10 HCL for 5 minutes.

- Colouring fuchsin acid and back for 15 minutes in a water bath at 90 ° C.

-Rinse with tap water.

Observation of stained sections, the sections were placed on slides, covered with a coverslip after dropping one or two drops of lactophenol, to prevent dehydration. Observations are made with magnification: x10, x40, x63 and x100, the microscope equipped with a camera.

Statistical Analysis

The results, presented as histograms often mean values; these have been made by the Excel software. In the case of paintings, the results subjected to statistical analysis (analysis of variance, ANOVA / MANOVA) with the help of STATISTICA software.

Results

Effects of mycorrhiza on the aerial and root part (macroscopic observation)

Characteristics of plants inoculated and uninoculated argan were determined monthly growth, a control of the root system after staining showed that uninoculated were not mycorrhizal while all plants were inoculated. The effect of inoculation (and therefore mycorrhiza) is very clear. Compared to non-mycorrhizal condition, mycorrhiza leads to nine months, under our experimental conditions, our statistical analysis shows that the differences in growth parameters between mycorrhizal and non-mycorrhizal plants are very highly significant (Fig 3)

Fig. 3: A. The argan non-mycorrhizal plants (controls); B. Plants argan mycorrhizal (inoculated).

The results show an increase of 70.5 ± 1.35 cm from the average stem height (Table I), with high average number of branches of 1st and 2nd order reach 23.71 ± 0.97 and 15.37 ± 1, 19 respectively. And 52.36 ± 1.08 cm in average root length, with high average number of secondary roots reach brown 32.56 ± 1.45 (Fig 4, Fig 5).

Fig. 4: Evolution of the aerial and root part (average length of stems and roots) of argan mycorrhizal and non-mycorrhizal plants for 9 months.

Fig.5: Evolution of argan mycorrhizal plants and non-mycorrhizal for 9 months (number of branches of 1st and 2nd order and number of secondary roots).

Similarly, ground biomass is increased by 11.45 ± 1.47 and root biomass of 7.47 ± 0.52 in plants inoculated (mycorrhizal) against by the non-inoculated control plants were recorded as the values of the low plants inoculated values of the average stem height, average number of branches of 1st and 2nd order, the average root length, the average number of secondary roots, respectively about 40.4 ± 0.44 cm, 07, 65 ± 0.71, 0, 35.58 ± 0.92 cm, 13.83 ± 1.04. Even the root and shoot biomass recorded values within the range of 7.53 ± 0.86 and 4.25 ± 0.48 respectively, the difference between the dry weight and mycorrhizal mycorrhizal plants are not significant for the root biomass is highly significant for aerial biomass (Fig 6),

Fig.6: Evolution of Biomass argan mycorrhizal and non-mycorrhizal plants for 9 months.

The index of relative mycorrhizal dependency (IRMD) is 45.77%. Under the effect of mycorrhiza, the average air ratios parties / party root password from 1.05 to 1.50, indicating a greater efficiency of mycorrhizal root systems (Fig 7).

The average plant size (height in cm), the average number of branches of 1st and 2nd order and the average number of secondary roots were recorded after 3, 6 and 9 months of growth for each treatment is shown in Figure 4. Whatever the date, the inoculated plants are always larger than the average non-inoculated plants.

Fig.7: IRMD Evolution of argan mycorrhizal and non-mycorrhizal plants for 9 months.

Microscopic Observation

The microscopic observation of root fragments of inoculated plants (mycorrhizal) light microscope shows a high density of nodules on the roots. It is probably endomycorrhizae represented only by spherical vesicles, intracellular hyphae and even platoons. These latter structures are encountered only in the nature in the family Endogonaceae, hence the name endomycorrhizae vesicular arbuscular (VA). Endomycorrhizal mycelium generally Zygomycetes enters host cells in which it is a branched platoon (Fig 8).

Fig.8 : A . Roots inoculated (pack intracellularG : X63) ; B. raciness infected (G × 5250) ; C. Morphology of vesicles (G × 2100).

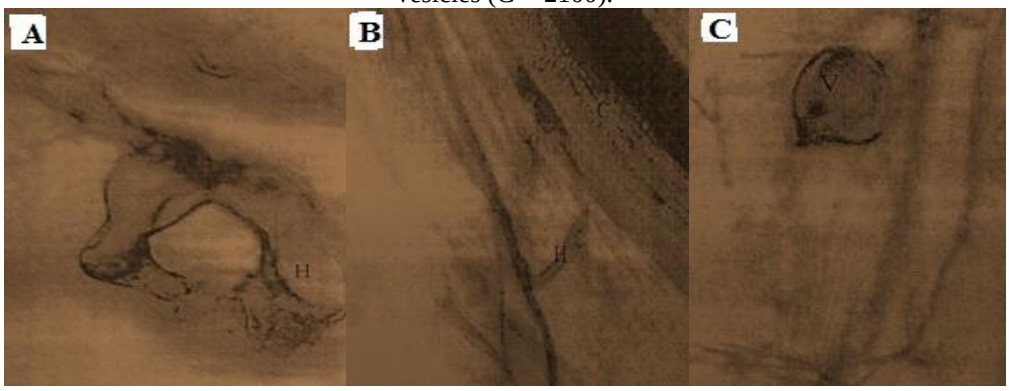


Table 1: Evolution of the aerial part of argan seedlings (height of the stem and branches).

parameters measured	Observation periods Seedlings argan mycorrhizal (days)								
	10	20	30	40	50	60	70	80	90
Average height of the stems of seedlings mycorrhizal (cm)	7,2±1,39 6,2±0,32	20,7±1,42 18,3±1,73	41,0±0,54 20,8±1,22	48,5±1,12 24,0±0,27	54,1±0,61 27,7±1,31	56,2±1,66 30,1±1,14	59,0±1,73 35,0±1,17	64,3±0,82 37,4±1,38	70,5±1,35 40,4±0,44
Average height of the stems of seedling controls (cm)									
Average number of branches of mycorrhizal seedlings (first order)	8,66±1,63 03,18±0,12	09,43±0,81 03,62±0,08	10,56±1,65 04,61±0,43	12,16±0,34 04,18±0,54	15,54±1,23 05,76±1,02	16,19±0,67 05,78±0,43	19,52±1,56 06,59±1,41	21,12±1,12 06,64±1,04	23,71±0,97 07,65±0,71
Average number of									

branches of the control plants (1st order)									
Average number of branches of mycorrhizal seedlings (second order) Average number of branches of the control plants (2nd order)	05,24±0,14 --	06,43±1,02 --	07,10±0,54 --	08,48±0,36 --	09,12±1,04 --	12,5±0,61 --	14,82±0,65 --	15,30±1,74 --	15,37±1,19 --

Table 2: Evolution of the root part of argan seedlings (primary and secondary root).

parameters measured	Observation period Seedlings argan mycorrhizal (days)					
	15	30	45	60	75	90
Average length of the main root of mycorrhizal seedlings (cm) Average length of the main root of control plants (cm)	8,82±0,54 6,74 ±0,64	14,15±0,73 8,12±0,62	40,9±1,12 27, 88±0,32	45,1±0,76 32,26±1,14	50,7±0,12 33,73±0,65	52,36±1,08 35,58±0,92
Average number of secondary roots of seedlings mycorrhizal (brown) Average number of secondary roots of control seedlings (color white)	17,29±0,68 10, 12±0,43	20,10±1,04 10,84±0,64	24,72±0,75 12,18±1,24	27,63±0,45 12,57±0,43	29,42±1,02 13,74±0,42	32,56±1,45 13,83±1,04

Table 3: Evolution of Biomass and IRMD of argan mycorrhizal and non-mycorrhizal plants for 9 months.

		After 3 months	After 6 months	After 9 months
Aerial biomass (g)	M^+	4,82±0,52	8,65±0,82	11,45±1,47
	M^-	3,57±0,6	5,08±0,28	7,53±0,86
Root biomass (g)	M^+	3,25±1,05	5,63±1,02	7,47±0,52
	M^-	2,85±0,48	3,75±0,18	4,25±0,48
dry weight M^+ (g)	M^+	8,07±1,57	14,28±1,84	18,92±1,99
dry weight M^- (g)	M^-	6,42±1,08	8,83±0,46	10,26±1,34
IRMD %		20,44%	38,16%	45,77%

Discussion

Response to mycorrhizal woody plants from seed has already been observed by other authors (Bâ et al. 2001 and Turjaman et *al*., 2006). The implementation of a large number of repeats allowed us to demonstrate a highly significant effect of the inoculation. Through various physiological mechanisms (Augé, 1996), mycorrhizal symbiosis can help young plants to cope with difficult conditions in arid zones (Nouaïm and Chaussod, 1996). The adaptive capacity of the inoculum soil environment, its extraradical development and its competitiveness effect by the indigenous microflora are important parameters (Caravaca et *al*., 2003). Finally, the use of an aerated substrate suitable for the growth and branching of the root system of the argan tree and the installation and development of mycorrhiza. We can expect these features have a positive effect on the rate of recovery and initial growth after transplantation (Nouaïm and Chaussod, 1997; Duponnois, 2007). The results clearly show a beneficial effect of mycorrhizal inoculation on plants grown from seeds of the argan tree, mycorrhizal root systems are always more effective than non-mycorrhizal, aerial biomass relative to root biomass was greater in the first . When the availability of phosphorus and trace elements is limited, mycorrhizal symbiosis is promoted and the growth of inoculated plants is faster than non-inoculated plants (Echairi, Nouaim and Chaussod, 2008). The results obtained in the tests mycorrhiza show a very positive effect on the growth and branching of the aerial part of seedlings argan. Indeed, the growth in height of the shaft has a significant increase. By cons, in control seedlings, the average growth during the same experimental period (9 months) is relatively low. The same results were also obtained in the same species by Ben chettouh (2000). According to the first results concerning the effect of mycorrhiza on the growth of the root part, it seems obvious positive effect on elongation and the number of youth-developed roots. So mycorrhizal roots show an increase in primary root length. By cons, in control plants, increased primary root length is very small. As for young people developed roots, it seems the positive effect of mycorrhiza. These results are in agreement with those of Hatimi and Lagbouri (1995). According Jay achandran et *al* (1992), the efficiency of mycorrhiza root system is mainly due to an extension of the surface of absorption and volume of soil explored thanks to fungal hyphae. The fungus provides the point of intimate contact with the ground by the thin extraradical hypha which extends to several millimeters from the root. Sylvia (1986) measured an average of 12 meters of hyphae of endomycorrhizal and considers that the length of hyphae that develops around the root can reach 100 to 200 meters to a centimeter root. This extensive hyphal network improves the absorption of water and nutrients. Roots observed always have the same type of mycorrhiza, the hyphae of variable diameter passes from cell to cell via a single hyphae and intracellular form platoons sometimes present in the outermost cortical cells, the infection never propagates in the central cylinder (the hyphae never enter into the vascular system). We also observed a fungal intracellular structure, more or less rounded. These structures are intracellular vesicles, the vesicles endomycorrhizal

product storage and within the root symbiotic cells when approaching senescence (Aller, 1992). These vesicles are storage organs rich in lipids and calcium and Reproductive. We also observed a highly branched structure called hyphal arbuscular inside root cells, infection creates an absorbent structure with a very large surface of the transfer of nutrients from the plant and the fungus (Sanders et *al*., 1997).

Conclusion

At the end of this work, it is possible to consider that the argan tree has very interesting ecological and physiological traits. It even is the tree of the future for the fight against desertification, soil protection against erosion, and provides a significant contribution of organic matter directly or indirectly, in its ecological interest, is threefold: forest, forage and fruit (oil production). The results of mycorrhiza in argan plants clearly show a beneficial effect of inoculation on plants grown from seeds of the argan tree, mycorrhizal root systems are always more effective than non-mycorrhizal, aerial biomass relative to root biomass is greater in plants inoculated. When the availability of phosphorus and trace elements is limited, mycorrhizal symbiosis is promoted and the growth of inoculated plants is faster than seedlings not inoculés.la mycorrhizal symbiosis can help young plants to face the harsh conditions of arid. The adaptive capacity of the inoculum soil environment. So the use of controlled mycorrhiza could probably significantly increase the success of rehabilitation projects that heritage assets, and reforestation outside the province of Tindouf.

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